immediate drainage area of the initial lesion.

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## Assessment of rapid method for identifying *Escherichia coli*

Most pathogenic enterobacteria identified in medical microbiology laboratories are *Escherichia coli*. If a simple, inexpensive, and rapid yet reliable method for identifying these isolates was available it would greatly improve efficiency. A kit recently introduced by API-bioMérieux (UK) Ltd, Rapidec coli, seemed to fulfil these requirements and we assessed its reliability based on results for 1000 *E coli* and 250 other enterobacteria isolated from urinary tract infections.

The Rapidec coli kits were supplied by the manufacturers (API-bioMérieux (UK) Ltd Basingstoke). Each organism is tested using four cupules designated C, S, 1 and 2; C is an opacity standard for the bacterial suspension which is prepared in S while 1 and 2 contain media for the detection of $\beta$ glucuronidase and $\beta$ galactosidase, respectively. The addition of James reagent (API-bioMérieux (UK) Ltd) to cupule 2 also detects indole production. Five groups of four cupules are combined on a plastic strip and any sets of four cupules not required can be cut off, refrigerated, and used the following day. Sterile distilled water is added to cupules C and S and the growth from two to three colonies of the organism to be tested is homogenised in cupules S to the same opacity as the standard; 50 $\mu$l of the suspension are then transferred to each of the cupules 1 and 2. After incubation at 37° for two hours a yellow colour in cupules 1 and 2 indicates a positive test for $\beta$ glucuronidase and $\beta$ galactosidase, respectively; in negative tests the suspension remains colourless. After reading these results one drop of James reagent is added to cupule 2 in which the development of a red colour is positive for indole production. If all three tests are positive the organism is regarded as *E coli*, with only two tests positive the organism may be *E coli* but further tests are required (indeterminate) and with only one positive or all negative the organism is not *E coli* (non-*E coli*).

Table *Reliability of Rapidec coli designations of enterobacteria from urinary tract infections*

| *Designation by Biochemistry*/API 20E* | *Rapidec coli* | | | |
|---|---|---|---|---|
| | *No of isolates* | | | |
| | *E coli* | *Non-E coli* | *Indeterminate* | *Total* |
| *E coli* | 897 | 3 | 100 | 1000 |
| Non-*E coli* | 0 | 212 | 38 | 250 |

*Based on Cowan[1].

The 1250 isolates were designated as *E coli* or non-*E coli* on the basis of standard biochemical tests[1]: the tests used included indole, MR/VP, citrate, malonate/PPA, urea, glucose, lactose and motility. If there was any doubt about the designation of any isolate, or this differed from that by Rapidec coli, then it was identified by API 20E as were all isolates designated as indeterminate by Rapidec coli; in these cases the definitive designation was determined by the API 20E result.

One thousand isolates were designated *E coli* by biochemistry or API 20E and 250 as non-*E coli* (*Citrobacter, Enterobacter, Hafnia, Klebsiella, Morganella, Proteus, Providencia* and *Serratia*); these designations, in relation to those derived from the Rapidec coli tests, are shown in the table. A designation of *E coli* by Rapidec coli was always correct. Three isolates were incorrectly designated non-*E coli* and in each case this was because the indole test was negative with Rapidec coli whereas it was positive by the standard method and by API 20E; two isolates were designated as indeterminate for the same reason. Of the 38 non-*E coli* designated indeterminate by Rapidec coli, 25 were *Klebsiella oxytoca*.

Rapidec coli was extremely easy and quick, both to set up and to read (average two minutes for an isolate). Used as a means of eliminating about 90% of *E coli* from more costly and time consuming identification procedures, it was absolutely reliable with these urinary enterobacteria, and by giving a result in two hours it caused no delay in obtaining a result for those isolates requiring further identification. The savings to be made in any particular laboratory, by incorporating Rapidec coli in an identification procedure, would depend on its cost in terms of time and material relative to that of the usual identification method and on the proportion of enterobacteria routinely identified as *E coli*. For example, if the cost of Rapidec coli is 20% of the usual method then savings would be made once the proportion of *E coli* exceeded 22%; at 50% and 90% *E coli* the savings would be 25% and over 60%, respectively.

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## Nucleolar organiser regions and proliferative index in glandular and squamous carcinomas of the cervix

Little is known about the pathogenesis of adenocarcinoma and adenosquamous carcinoma of the cervix. The frequent association of adenocarcinoma in situ with squamous intraepithelial and invasive neoplasia, and the occurrence of mixed adenosquamous lesions, both in situ and invasive, suggests the possibility of common aetiological factors between glandular and squamous tumours. Although some larger studies have found no difference in survival

between adenocarcinoma and squamous carcinoma of the cervix,[1] more recent work has suggested a rise in incidence of mixed invasive adenosquamous carcinoma in young women which may be associated with a higher incidence of nodal metastases compared with other histological types of cervical carcinoma.[2,3]

Nucleolar organiser regions (NOR's) are chromosomal segments containing encoded ribosomal RNA which can be visualised in histological sections using a silver colloid technique when they are termed AgNOR's. We looked at AgNOR's in the different histological types of cervical carcinoma and compared them with the proliferative index as determined by flow cytometry, with the aim of determining whether the adenosquamous tumours represent a separate and more aggressive subtype.

We applied the silver colloid method[4] to 46 carcinomas comprising 19 squamous, seven squamous with occasional intracellular mucin globules, 15 adenosquamous and four adenocarcinomas, and counted 200 randomly selected cells in each case. Each tumour was graded as I, II, or III by a separate observer, corresponding to well, moderately, or poorly differentiated, respectively. Flow cytometry was performed on 24 cases, using tissue retrieved from stored paraffin wax blocks, and stained with propidium iodide. The proliferative index (PI) was calculated as the proportion of cells in the DNA synthetic phase. Only three cases were aneuploid, all were adenosquamous in type and of grade II (n=1) or III (n=2) (table).

The large standard deviation within some groups highlights the wide spread of results between cases. Differences between the groups were analysed using Student's *t* test. There was no significant difference in the proliferative index among any of the tumour types. There was no difference in the AgNOR count between the squamous, squamous with mucin, and adenosquamous tumours. The difference in AgNOR count between adenocarcinomas and both squamous and adenosquamous carcinomas just reached statistical significance ($t=0{\cdot}011$ and 0·042, respectively, $p < 0{\cdot}05$). Overall, there was no association between tumour grade and either AgNOR count or proliferative index, and, interestingly, no correlation between proliferative index and mean number of AgNOR's per cell.

We conclude that enumeration of AgNOR's is of no practical use in distinguishing between the histological type or grade of cervical carcinoma, and that the AgNOR count does not seem to be a direct marker of proliferative activity in these tumours taking flow cytometric determination of proliferative index as the baseline. The AgNOR technique did not show any difference between adenosquamous tumours and the other types to suggest that adenosquamous tumours are a more rapidly progressing type. Similarly, the proliferative index does not suggest this, but the fact that all aneuploid tumours seen were in the adenosquamous group merits further study. The most important use of these methods would clearly be in predicting survival in an individual case, and further work to evaluate their possible use for this is in progress.

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Table *AgNOR count and proliferative index in different histological types and grades of cervical carcinoma*

| | *AgNOR's* | | | *Proliferative index* | | |
|---|---|---|---|---|---|---|
| *Tumour type/grade* | *No tested* | *Mean AgNORs* | *SD* | *No tested* | *Mean PI (%)* | *SD* |
| Squamous | 19 | 5·99 | 1·89 | 6 | 16·27 | 6·85 |
| Squamous with mucin | 7 | 5·84 | 2·21 | 3 | 23·17 | 8·98 |
| Adenosquamous | 15 | 6·04 | 2·43 | 10 | 28·21 | 13·78 |
| Adenocarcinoma | 5 | 3·58 | 0·77 | 5 | 31·81 | 26·45 |
| Grade I | 4 | 4·16 | 1·03 | 4 | 29·87 | 23·30 |
| Grade II | 25 | 6·04 | 2·03 | 11 | 25·63 | 16·41 |
| Grade III | 17 | 5·64 | 2·38 | 9 | 22·98 | 12·50 |

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# Matters arising

## Granulomas of the kidney induced by Bacillus Calmette Guerin (BCG)

We read with interest the recent letter describing granulomata of the prostate induced by Bacillus Calmette Guerin (BCG).[1] We saw similar granulomas in a nephrectomy specimen from a 73 year old man who had received intravesical BCG for bladder cancer. An incidental finding was a wedge-shaped lesion related to a dilated calyx and containing both caseating and non-caseating granulomas. The patient had no evidence of tuberculosis clinically or on subsequent investigation. He almost certainly had vesicorenal reflux, and this probably gave rise to the granulomas. Full details of this case have been submitted to the *British Journal of Urology*.

Two other cases of renal granulomas following intravesicular BCG have been described.[2,3] These cases, together with the prostatic examples reported by Ramani and Griffin, illustrate that we can expect to find such granulomas anywhere within the urinary tract. These are likely to become more common as the use of BCG in treating bladder cancer becomes more widespread.

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